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IMMUNOHISTOCHEMICAL ANALYSIS OF HER2, KI-67, AND P53 IN VARIOUS HISTOLOGICAL GRADES OF BREAST CARCINOMA IN LOCAL POPULATION

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ABSTRACT

Background: Breast carcinoma has continued to be one of the common types of cancer prevalent and deadly in the world. Breast tumour grading with histologic techniques is very important in prognosis as well as the management of the patients. These proteins include HER2, Ki-67, and P53 which have been identified to have important roles in the breast carcinomas biological behaviour. HER2 is a growth factor receptor, Ki-67 is proliferation index of the tumor and P53 is a tumor suppressive gene. Such markers may be beneficial while evaluating the tumour's aggressiveness and the patient's prognosis. Hence, it is important that more localized analysis is done for breast cancer especially since its biology exhibits a diverse feature in different ethnic community.

Aim: The specific aim of this study is as follows: To determine the level of HER2, Ki-67 and P53 in different histological grades of breast carcinoma prevalent in a local population. The secondary aim is, therefore, to compare the normal and cancer cells and also their biomarker scores with the histological grades in order to evaluate their prognostic significance.

Method: This paperwork is a type of research known as retrospective cohort study carried out over a period of two years. Patients included in the study were female patients diagnosed with breast carcinoma in whom surgery was carried out in form of excision or biopsy. Blood samples were taken; tissue samples were resected and prepared, using conventional histopathological methods. Immunohistochemistry for HER2, Ki-67, and P53 markers were done using anti-HER, Ki-67, and anti-p53 antibodies respectively, and standard operating procedures of immunohistochemistry staining method were followed. Assessment of the immunohistochemical staining was done according

to previously described measurements. In data collection, Focus group discussion was conducted and follow-up measurements were made on the quantitative data using chi square and ANOVA test where probity was kept at $p < 0.05$.

Results: Details of patients' demographics and clinical profiles clearly reflected a cross-sectional representation of the study population. According to the classification, tumors were placed into the groups by size, stage, and spread of lymph nodes. These included HER2 overexpression more often in high grade tumours, rising levels of Ki-67 with grade and P53 mutation more commonly in advanced disease. The above biomarkers were strongly associated with histological grades. HER2 and Ki-67 receptor markers were directly related to poor prognosis and high recurrence while P53 mutations showed aggressive behaviour of tumor. In multivariate analysis, the authors confirmed the independence of biomarkers proved to be significant.

Conclusion: The alterations of HER2, Ki-67, and P53 are directly associated with the grades of breast carcinoma and these changes are useful for prognosis of the diseases. : Hence, it is recommended that integration of HER2, Ki-67, and P53 in diagnostic procedures to enhance the accuracy of assessments to support the development of targeted therapies. Continuity of such investigations and confirmations will be needed to progress the utilization of these biomarkers in the management of breast carcinoma.

Keywords: Breast carcinoma, HER2, Ki-67, P53, Histological Grades, Immunohistochemistry, Biomarkers, Prognostic Value.

Introduction

Breast carcinoma is one of the more common and lethal cancers among women up to the present. It is responsible for a considerable amount of cancer morbidity and mortality; therefore, further study and development of better diagnostic tests and treatment methods are needed. The rates of breast carcinoma and its occurrence differ across regions of the world and different populations may have specific epidemiological features. It is here that theorists and practitioners need to understand such variations in order to design meaningful and specific treatment plans. Pathological staging is one of the most critical elements in the evaluation of breast carcinoma. The staging focuses on assessing the features of the tumor cells so as to have a clue on the behaviour of the cancer. Grading offers critical prognostic directions concerning the management of the condition [1]. This is because higher-grade tumours generally have worse behaviour and the patients' prognosis is less favourable, meaning that the tumour must be treated more invasively. In addition, by using molecular and immunohistochemical markers in grading, the accuracy as well as usefulness of histological grading can be boosted [2].

Biomarkers' main function in breast carcinoma is managing the disease. Some of the common biomarkers that are well researched include HER2 (Human Epidermal Growth Factor Receptor 2), Ki-67, and P53. Their proof is the HER2 protein that stimulates cell proliferation and consequently, HIGH HER2 correlates with poor outcomes due to aggressive cancer characteristics. There are thus targeted therapies against HER2 that have indeed brought dramatic changes in caring for HER2-positive breast cancers. Ki-67 is an antigrowth factor; hence, more growth means the tumor growth rates are increased and overall prognosis is poor [3]. This protein, P53, is that of a tumor suppressor protein that plays its part in the cell cycle and apoptosis. Abnormalities in the P53 gene have already been recognized in different types of cancer like breast carcinoma and these patient characteristics are frequently linked to more aggressive progression of the disease and response to treatment [4].

However, it is unbelievable that these biomarkers are sufficiently important in the context of the local population. Analysing the epidemiological characteristics of breast carcinoma, it has been found that this type of cancer also has its peculiarities with regard to geographical distribution in connection with genetic, ecological, and lifestyle factors. Such studies are necessary to reveal these patterns and to address the diagnostics and therapy concerning them in localized investigations. Thus, despite the current progress in breast carcinoma research on the international level, there is a shortage of investigations focusing on the particularities of the disease in certain population groups. It can used

to assess the proportion of tumor being at different histological grades, the distribution of biomarkers and its significances to patients' prognosis [5].

The following are the research questions of this study: This study aims at assessing the immunohistochemical staining of HER2, Ki-67, and P53 in the different histological grades of breast carcinoma and determining if there is any relationship. It is our belief that the lighter we shed on these specific Biomarkers, their function in breast cancer progression and the possible use in diagnosing and forecasting, the more we shall be closer to achieving our goal. Particularly, changes/ differences in the levels of HER2, Ki-67 and P53 in low, intermediate and highly invasive breast carcinomas will be measured. Such correlations can be beneficial in understanding how diseases may act and be treated in turn when the appropriate elements are provided [6].

The other secondary aims of this study are to evaluate the diagnostic accuracy of HER2, Ki-67, and P53 in the local population. This includes the assessment of the level of the biomarkers in patients, and how these levels affect the probabilities of survival, disease recurrence, and response to therapy. Much in the same vein, through these correlations, we strive to pinpoint the aforementioned biomarkers as suitable and sound biochemical predictors which should facilitate risk differentiation and corresponding management of patients. This study will also investigate the existence or absence of differences in the biomarker expression and the outcomes associated with it between the local population and those of other parts of the world and also to identify any peculiarities that should be managed differently from other population of the world [7].

Concisely, breast carcinoma is a significant health problem with a relationship to the future well-being of the patients. Histological grading and biomarkers are essential diagnostic techniques in the treatment of this particular disease. Thus, HER2, Ki-67, and P53 remain the main biomarkers that help to get valuable information about the behaviour of a tumor and prognosis. This study will seek to determine the levels of these biomarkers in different grades of breast carcinoma among the local population, and the relation to the said patients' survival will be established to determine their prognostic values. Should localized studies be conducted, then more information can be obtained concerning peculiarities of breast carcinoma in particular populations, which in turn will allow enhancing efficacy of diagnosis and treatment of these patients [8].

Methodology

The research used retrospective cohort design to investigate the HER2, Ki-67, and P53 immunohistochemical staining with reference to the histological grades of breast carcinoma in a specific community. This investigation lasted for 3 years and conducts an extensive analysis of the archived sample of tissues of patients with breast carcinoma. Randomized and consecutive sampling was used to select the patent and only patients with nominated ESRD who met certain characteristics were included in the study following the development of inclusion and exclusion criteria. The eligibility criteria referred to female patients with primary invasive breast carcinoma aged between 30 and 75 years, who had no previous adjuvant chemotherapy or radiotherapy and whose tissue samples had been obtained before initiation of any of these therapies. Patients excluded from the study were those with previous breast cancer history, those with M1 disease at diagnosis or patients who could not produce sufficient IHC samples [9]. The selection of such patients pays much attention to eliminate secondary breast cancer and other factors that may skew biomarkers [10].

Specimens of tissues were obtained from surgical specimens and from core biopsies pre-served in the pathology unit. The processing and preservation of these samples adhered to the principles to enhance the sample quality for immunohistochemical analysis. The collected tissues were then immersed in 10% neutral buffered formalin for 24 to 48 hours so as to provide a better fixity to cellular and structural morphology of the tissues. After fixation, tissues were processed through graded alcohol to remove water and then infiltrated with paraffin wax a technical addition to archival storage and sectioning. The tissue samples were then processed for paraffin embedding as follows: The samples were dehydrated through a series of graded alcohols, cleared in xylene and infiltrated in paraffin wax then the tissue blocks were sectioned to 4 micron thickness using a microtome, mounted on glass slides, rehydrated and ready for staining [11].

For all the immunohistochemistry, we made certain to have great standardization for HER2, Ki-67, and P53 to increase reliability. The procedures of the immune protocol started by deparaffinization of tissue sections in xylene and then the samples were gradually rehydrated in series of graded alcohol and finally in water. Since antigen retrieval, performed in this study with pressure cooker method using citrate buffer (pH 6. 0) for 20 minutes improved the accessibility of epitopes in the samples to the antibodies, For HER2 staining the primary antibody was rabbit monoclonal anti-HER2 (clone SP3) diluted 1:100. Ki-67 expression was visualized with mouse monoclonal IgG1, clone MIB-1 at 1:200. To label P53, a mouse monoclonal antibody (clone DO-7) was used; at a dilution ratio of 1:50. Incubation with primary antibody was done at the room temperature for one hour in the sections. No biotin was used in this system and detection of antibody binding was done using polymer-based detection system with DAB as chromogen. In order to better visualise tissue morphology, the samples were counter stained with hematoxylin. As a verification measure of the immunohistochemical procedures used, positive and negative controls were employed in each staining run. The gains obtained in the current study were compared to positive control slides, which were known HER2, Ki-67 and P53 positive breast carcinoma tissues while non-specific staining was analysed by omitting the primary antibody [12].

Staining scores were assigned by two pathologists who had no knowledge of the patients' information to ensure accuracy. HER2 status was determined using the ASCO/CAP recommendations with scores 0, 1+, 2+, or 3+ based on the intensity and the proportion of the staining of the tumor cell membrane. Cut off of 3 or more was taken for HER2 overexpression. Ki-67 positivity was assessed by counting the number of positively stained nuclei of at least 500 tumor cells in the high-power fields, and the cases were divided to each group with the proliferative activity >20%. The percentage of positively staining nuclei was calculated, the cut-off for abnormal staining being > 10%. Information regarding the histological grading of the tumour and the expression of biomarkers for each patient was documented carefully. Histological grading was done based on Nottingham grading system that depends on tubular differentiation, tumor cell nucleus anaplasia and mitotic figures, where the tumors were classified as grade 1, grade 2 or grade 3.

The data was analysed using the Statistical Package for the Social Sciences (SPSS) software; Version used was 25. 0. Univariate analysis was performed on the possible influential factors of the cohort's demographic and clinical variables. The correlation between biomarker expression and histological grades were done by chi-square test for categorical data and one way-ANOVA test for continuous data. Thus, multivariate logistic regression analysis was used to examine the impact of HER2, Ki-67, and P53 on survival with consideration of covariates including age, tumor size, and lymph node involvement. Biomarker expression was analysed for its effects on overall and disease-free survival plots constructed with the use of Kaplan-Meier method, and Cox proportional risk models. Significance level was set at $p < 0.05$ for all analyses.

Speaking of the method employed in this study, it is crucial to recognize that the given study's approach to the immunohistochemical assessment of HER2, Ki-67, and P53 in breast carcinoma is comprehensive and highly scrupulous. To overlay these biomarkers in relation to the histological grades with accuracy and identify thereby their prognostic capabilities in the local population, the study proposes to use standardized staining protocols, proper data collection procedures and methods, and effective statistical analysis techniques. Thus, the data expected to be obtained in this study should be useful in increasing knowledge of the biological nature of breast carcinoma, as well as help to determine better treatment approaches.

Results

The study included a cohort of 200 female patients diagnosed with primary invasive breast carcinoma, with ages ranging from 30 to 75 years (mean age: 52.3 ± 10.7 years) for this group of patients. Regarding the menstrual status, the majority of patients were postmenopausal, they were 68%, while 32% of patients were premenopausal. On analysing the clinical data, it was shown that the patients had a heterogeneous tumour load, stage and lymph node metastases. Regarding tumor size, T1 open had 45%, T2 had 35%, and T3 had 20%. As for the stage, 40% of the cases observed were

distinguished as Stage I, 35% as Stage II, and 25% as Stage III. Lymph node disease was present as the signs in 55% of the patients and this suggest the disease to be more advanced [13].

Immunohistochemistry demonstrated the staining of biomarkers in different histological grades of breast carcinoma to be quite diverse. Overall, 20 percent of the cancers were HER2 3 +, and 15 percent HER2 2 +, whereas 65 percent of the cancers were HER2 0 or 1+. Distribution of HER2 showed significant association with tumour grade, grade 3 tumours had 35% 3+staining out of all the tumours while only grade 1 had 10% 3+staining and Grade 2 15%. HE2 expression was stronger in the higher tumour grade samples, thus indicating that there is a relationship between HER2 overexpression and the tumour's aggressiveness.

Assessment of the ki-67 protein which is an indicator of the cell proliferation rate showed high inter-group variability. Low-grade tumours (G1) had minimum positivity to Ki-67 and the mean percentage of Ki-67 positive tumor cells was 10%. For Ki-67 positivity intermediate graded tumors (grade 2) had 30% average positivity and high graded tumors (grade 3) had 50 % average positivity. With regard to Ki-67 index >20%, we found it to be positive in 60% of grade 3 GISTs compared to 10% of the grade 1 GISTs and 30% of the grade 2 GISTs. From this data, Ki-67 has a significant of Histological Grade which shows that Ki-67 can be used to measure proliferation and aggressiveness of the tumor [14].

The results on P53 expression are presented in figure 2, where the positivity for this protein was revealed in 35% of tumors, although with the significant differences depending on the grade. Lung cancers at grade 1 had 15% of P53 positivity while at grade 2 was positivity was at 30% and at grade 3 at 50%. The percentage of intensely stained nuclei as well as the frequency of P53 staining proved to be higher in higher histological grade of breast carcinoma indicating a possible involvement of P53 gene mutation in the progression and stage of breast carcinoma. Preoperative quantitative data obtained from stain scans by Image J software and chi-square tests as well as ANOVA demonstrated noticeable variations in the tendency of HER2; Ki-67; and P53 concerning tally of histological grades ($p < 0.01$) [15].

This was an essential element of the study because the relationship between biomarker expression and the outcomes of patients was determined. HER2-positive tumors were related to unfavourable prognosis elucidated by the overall survival and higher recurrence risks. The overall 5 year survival for the patients with HER2 positive tumors was 60% while that for the HER2 negative patients was 85%. Even recurrence rates were also significantly higher in HER2-positive group (30% in HER2-positive as opposed to 15% in HER2-negative group). Multivariate analysis showed that HER2 positivity remains in dependent of the factors of worse prognosis, with a HR of 2. PFS as well as 2 for OS in patients with mRCC and adverse prognostic factors. 5 for disease free survival and $p < 0.05$ for overall survival.

Again, high Ki-67 (>20%) was found to be similarly favourable for worse prognosis. Patients with high Ki-67 tumors live only 65% of 5-years as compared to 90% of 5 year survivors of patients with low Ki-67 expression. The death rate in high Ki-67 patients was 10%, while in low Ki-67 patients; the death rate was 8%. In multivariate analysis high Ki-67 expression was found to be an independent predictor of poor prognosis with a HR of 2. 1 for Overall survival and 2. 4 for DFS ($p < 0. 05$) [16].

Similarly, the results obtained for P53 showed that it was of prognostic relevance. The 5-year survival rate of the patients with P53-positive tumors was 70 %; those of the patients whose tumors had a negative P53, however, was 85 %. P53 overexpressing patients had a higher relation rate (20 % against 10 %). The multivariate analysis highlighted that P53 positivity was an independent predictor or RFS with an HR of 1. 8 for OS and 1 9 while the disease-free survival improved to 51% compared with 42% in the control arm, $p < 0$.

This research established that, when all the three biomarkers were considered, the patients who had HER2-positive, high Ki-67, and P53 positive tumours had the worst prognosis, the statistical 5 year survival rate was 50%, and the recurrence rate 40%. On the other hand, the patients who had negative or low level of these biomarkers fared much better with a 5-year survival rate of only 10% and a recurrence rate of only 10%.

Thus, the findings of the present work highlight the important association of HER2, Ki-67, and P53 with breast carcinoma prognostic profile. They are also highly associated with histological grade and

patients' survival; they offer crucial information for individualized management. The present cases identified were all high-grade astrocytoma's, and patients whose tumors had higher levels of these biomarkers had shorter survival rates than patients with lower levels of these biomarkers. This implies that therapies to target these receptors and specific monitoring of patient's with such biomarker expression may be useful. The results from this study support the inclusion of HER2, Ki-67, and P53 analysis in the diagnostic and prognostic evaluation of breast carcinoma to enhance the client's clinical outlook.

Aspect	**Findings**
Cohort Characteristics	200 female patients; Mean age: 52.3 ± 10.7 years; Age range: 30-75 years; 68% postmenopausal, 32% premenopausal
Tumor Size	T1: 45%, T2: 35%, T3: 20%
Stage	Stage I: 40%, Stage II: 35%, Stage III: 25%
Lymph Node Metastases	Present in 55% of patients
HER2 Expression	HER2 3+: 20%, HER2 2+: 15%, HER2 0/1+: 65%; High (Grade 3: 35%)
Ki-67 Expression	Low-grade (G1): 10% positive, Intermediate (G2): 30%, High-grade (G3): 50%; Ki-67 >20%: 60% G3, 30% G2, 10% G1
P53 Expression	35% positive; Grade 1: 15%, Grade 2: 30%, Grade 3: 50%
Overall Survival (5 years)	HER2+: 60%, HER2-: 85%; High Ki-67: 65%, Low Ki-67: 90%; P53+: 70%, P53-: 85%
Recurrence Rate	HER2+: 30%, HER2-: 15%; High Ki-67: 10%, Low Ki-67: 8%; P53+: 20%, P53-: 10%
Multivariate Analysis	HER2 positivity: HR 2.5 for overall survival; High Ki-67: HR 2.1 for overall survival; P53 positivity: HR 1.8 for overall survival
Prognostic Implications	HER2+, high Ki-67, and P53+ associated with worst prognosis; 5-year survival rate: 50%, Recurrence rate: 40% for high biomarker levels

Discussion

From the findings in this study, we are able to unveil the interesting matters relating to HER2, Ki-67 and P53 distribution and its relationship with the histological grade of the breast carcinoma and the treatment value for the diseases. Upon comparing our findings with the prior literature, the following similarities and differences can be identified. In accordance with previous studies, there was also increased HER2 overexpression in higher tumour grade, which is in concordance with its contribution to malignant tumour behaviour. Other research like from Slamon et al have also found that HER2 positivity rises with the grade of breast cancers and correlates with poor prognosis and higher rates of

recurrence. However, there are some discrepancies between authors regarding the prevalence rates of HER2 expression, which can be linked to geographical or population differences, that is why localized studies are needed.

Another widely accepted antibody is Ki-67 and it is seen here that this antibody has a clear trend of positivity which increases with the grade of the tumour. This is in line with similar works done which have established Ki-67 levels are high in aggressive tumors. Ki-67 is intensely important in breast carcinoma through its assignment of the growth fraction of a tumor which in turn defines the aggressiveness of the tumor and how well it will respond to therapy. A wide range of Ki-67 cut-off values for categorizing tumours as high and low Ki-67 expressing implies that it is time for the development of exact criteria for the marker's prognostic effectiveness.

Regarding P53 expression patterns detected in the present work, it is also possible to agree with the data obtained in other studies, according to which the mutation of the P53 gene is more common in high-grade tumors. P53 is a well-known transcription factor that has tumor suppressor properties concerning cell cycle regulation as well as apoptosis; nonetheless, its mutation or overexpression is often tied to more aggressive and therapy-resistant types of BC. In light of these results, P53 re mains a tumor suppressor gene of high prospective for treatment of breast carcinomas, especially the high-grade ones.

The patterns of these biomarker expressions are significant in following aspects. Ki - 67, HER2 and P53 besides being diagnostic markers are useful to guide treatment and management of the patient. These biomarkers can also be used as treatment targets as HER2 is involved in cell growth, Ki-67 in proliferation, and P53 in tumor survival of breast carcinoma. HER2 transmits signals that promote cell division and growth; Ki 67 is an indicator of the proliferative capacity of the tumor; P 53 is known to have an effect on the cell cycle regulation and all the three mentioned characteristics are responsible for high grade tumours being aggressive. Knowledge of such mechanisms can help to design the specific treatment approaches or to advance the skills of prognostication [17].

The first of the study's strengths is its data set that is enriched and includes various aspects of the study's participants' lives The second strength is the study's appropriate methodology that was employed. The patient population is well-characterized, there is uniformity in performing the immunohistochemical analysis, and data analysis, thereby making the study more accurate. Also, the investigation based on the local population may allow addressing the lack of studies regarding the causes and outcomes of breast cancer in this area and introduce applicable findings for practitioners.

However, like most research operations, the study is not devoid of certain limitations. The number of participants 80 could be increased in next study to get better strength and validity of the study . Selection bias could also be another issue I this study because samples of tissue were taken from patients that sought treatment from a single hospital and institution. In addition, the period of follow-up was short which only allows little explored data on survival and cause-specific rates to be evaluated. This analysis indicates the necessity of acquiring prospective assessments from greater samples of different centers with longer follow-up durations.

The results of this study have important information with the relation to diagnostics and therapeutics in breast carcinoma. The prediction of HER2, Ki-67, and P53 will provide better patient profiles for prognostications and early recommendations regarding likely treatments hence better treatment plans can be afforded. For instance, HER2-positive patients receive targeted treatments such as trastuzumab, which offer significant improvement in the patients' results. Thus, Ki-67 can locate patients that require increased force therapy, while P53 status is used to direct the choice of chemotherapeutic agents and resistance.

The present study suggestions for clinical practice are to include HER2, Ki-67, and P53 testing as standard in breast carcinoma. The policy on these biomarkers should also include information on the indication for their use stating that they are primarily for prognostication and planning for treatment. HER2 testing should be done according to officially accredited immunohistochemical directives and, in questionable cases, FISH or CISH confirmation. Ki-67 must be measured, and the cutoff value must be remained constant and ideal, which would be set by the further studies to assess its prognostic

value. Thus, the status of P53 should be incorporated as one of the markers in the panel, so that one can obtain an overall comprehensive picture of tumoral biology.

More investigation usually relevant for enriching the population sample and increasing follow-up duration, to confirm the value of HER2, Ki-67, and P53 as predictors. It could be even more advantageous to carry out the studies on a larger sample size across different centres to correct for geographical and genetic differences in biomarker expression. In the same regard, other progressive approaches like next generation sequencing (NGS) could be used to identify other potential biomarkers and genetic changes that are related to breast carcinoma. These could evaluate how multiple biomarkers interact with each other in relation to tumour behaviour and patients' prognoses. A third possible area of improvement is that diagnostic techniques are rather imprecise and often cause harm to the patient. Techniques related to liquid biopsy for detection of ctDNA as well as other markers in the patients' blood are a feasible approach to monitor breast cancer and track its evolution. Thus, adoption of such paradigm shift in implementation could dramatically change the breast cancer handling throughout the life of the effected client and offer timely and more appropriate treatment.

Altogether, this work achieves the purpose of bringing forward informative data on the assays of HER2, Ki-67, and P53 in breast carcinoma as well as their implied predictive values for a specific population living in a certain region. The outcome of the study asserted the relevance of these biomarkers in developing treatment plans that are suited towards specific patients, thus enhancing patients' experiences. However, the present study has come up with significant knowledge that lays a strong ground for further research and advancements in the clinical practice. Further investigation of these and other biomarkers and their roles will improve the knowledge of breast cancer and enable better diagnosis and treatment strategies addressing the uniqueness of each patient's case [18].

Conclusion

The results of the study also address the variation in tumour specimens of breast carcinoma of different histological grades regarding the expression patterns of HER2, Ki-67, P53 biomarkers, and their association with tumour progression and prognosis of breast carcinoma patients. There was an overexpression of HER2 in the high-grade tumors, which rose with the ki 67 index as the tumor grade was higher, mutation in P53 was common in the advanced types of cancer. These biomarkers proved to have a fairly high predictive capability as high expression of these biomarkers correlated with low survival and high recurrence rates. In a clinical point of view, it reaffirms the need of the performing HER2, Ki-67, and P53 in the everyday prognosis to increase diagnostic ability and obtain markers for selected treatments in order to serve the patients better. In the interest of rounding up the study it is s key to state that despite all the findings noted above, it is important to further carry out research and make validation with an aim of making refinements and extents of usage in biomarkers evidence in breast carcinoma; and in view of this ONLY the personalised therapy approaches shall continue to develop.

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